TaffiX® nasal powder spray forms an effective barrier against infectious new variants of SARS-CoV-2 (Alpha, Beta and Delta)

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Short Report

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Abstract

While vaccination efforts against SARS-CoV-2 around the world are ongoing, new highly infectious virus variants continue to evolve. The protection provided by the available vaccines against some of the new variants is weaker. Additional preventive measures will therefore be needed to protect the population until effective vaccinations are widely available. *TaffiX®* is an anti-viral nasal powder spray comprised of low-pH hypromellose, which forms a protective mechanical barrier that prevents viruses from engaging with nasal cells. The current study aimed to test the protective effect of Taffix against Alpha (B.1.1.7; hCoV-19/Israel/CVL-46879-ngs/2020), Beta, (B.1.351; hCoV-19/Israel/CVL-2557-ngs/2020) and Delta (B.1.617.2; hCoV-19/Israel/VVL-12806/2021), three highly infectious and pathogenic SARS-CoV-2 strains. A nylon filter was treated with *Taffix®* gel, after which SARS-CoV-2 Alpha, Beta or Delta was seeded. After a 10-min incubation, the downstream side of each filter was washed, and the rinse was collected and placed over Vero-E6 cells. After 5 days of incubation, viral RNA was extracted and subjected to SARS-CoV-2 RT-PCR analysis. *Taffix®* fully blocked passage of all three tested SARS-CoV-2 variants, as demonstrated by a 100% reduction of recoverable viral RNA from Vero-E6 cells treated with filter rinse. These results support its use as an effective barrier against new variants of SARS-CoV-2 in conjunction with other protective measures.

Introduction

While vaccination efforts against SARS-CoV-2 around the world are ongoing, new highly infectious virus variants continue to evolve. The protection conferred by the available vaccines against some of the new variants is weaker [1], and experts are concerned that newer as well as yet undescribed variants of this RNA virus will eventually prove resistant to the current vaccines [2,3]. Additional preventive measures will therefore be needed to protect the population until effective vaccinations become available.

It is now well established that the nasal epithelium, specifically goblet and cilia cells, is the primary portal of entry of SARS-CoV-2 into the body, and protection at the nasal cell level could therefore be meaningful in reducing the risk of infection [4-7]. *Taffix®* is an anti-viral nasal powder spray comprised of low-pH hypromellose that upon insufflation into the nose, creates a thin gel layer over the nasal mucosa, forming a protective mechanical barrier, stable for at least 5 hours, that prevents viruses from engaging with nasal cells [8]. The acidity of the gel enhances the barrier by inactivating the viruses trapped within it. *Taffix®* is fully biocompatible according to the requirements of the current ISO 10993-1, ISO 14971, and FDA Biocompatibility Guidance (reports available upon request). It is commercially available in many countries across Europe, Asia, America and Africa. In a preclinical study testing its barrier performance when applied to a nylon filter, *Taffix®* effectively prevented passage of SARS-CoV-2 Hong Kong/VM20001061/2020 through the filter, as measured by plaque assay and viral RNA assessment of the flow-through [8]. In addition, in a prospective two-week user survey completed by 243 individuals participating in a two-day mass-gathering event in a highly endemic community, none of the 81 individuals who regularly applied *Taffix®* nasal spray during the event and the subsequent two weeks

reported on SARS-CoV-2 infection [9]. In contrast, of the 18 individuals who tested SARS-CoV-2-positive in the subsequent two weeks, 16 (98%) did not use *Taffix®* and the remaining two individuals reported using it once or twice in the study period. No side effects were reported.

The present in vitro study aimed to assess the protective effect of *Taffix®* against new pathogenic, highly infectious SARS-CoV-2 variants [10, 11], i.e., the Alpha (B.1.1.7; hCoV-19/Israel/CVL-46879-ngs/2020), Beta (B.1.351; hCoV-19/Israel/CVL-2557-ngs/2020) and Delta (B.1.617.2; hCoV-19/Israel/VVL-12806/2021) variants.

Results

The *Taffix®* layer applied to a cell strainer fully (100%) protected Vero-E6 cells from infection by the Alpha, Beta and Delta SARS-CoV-2 variants. Initial concentrations of approximately 300,000 to 600,000 copies/ml, which is equivalent to a clinical PCR result of CT21 to CT20, typically found in COVID-19 positive nasal swabs, were applied to the filter, with or without the *Taffix®* layer. No infection (zero) was diagnosed in Vero-E6 cells incubated with the solution used to rinse the downstream side of the Taffix®-coated filter. In contrast, Vero-E6 cells treated with medium used to rinse filters without *Taffix®*, showed very high Alpha (2,236,550 copies/ml; Figure 1), Beta (506,578,602 copies/ml (Figure 2) and Delta (54,583 copies/ml (Figure 3) virus copy numbers. The number of Delta viral RNA copies in the rinse solution eluted from *Taffix®*-treated filters was found to be 22.3-fold lower than in unprotected filters (60 copies/ml vs. 1338 copies/ml, respectively).

Discussion

In a prior in vitro study, *Taffix®* nasal spray powder formed a protective barrier limiting entry of SARS-COV-2 Hong Kong/VM20001061/2020 and preventing >99.9% of viruses from infecting Vero-E6 cells [8]. The protective effect of *Taffix®* in vitro was further verified by a real-life survey conducted in Israel during September 2020, following a “superspreader” event, which found a 78% lower rate of COVID-19 cases among *Taffix®* users as compared to those who did not use it at all or who failed to use it as recommended [9].

According to the American Centers of Disease Control and Prevention (CDC) [12] and the United Kingdom (UK) government publication [13], a variant called B.1.1.7 with a large number of mutations was detected in the Fall of 2020. This variant spreads more easily and quickly than other variants. Recent data from the UK reported that this variant may be associated with an increased risk of death compared to other variant viruses [14]. It has since been detected in many countries around the world. This variant was first detected in the US at the end of December 2020 and is now the dominant variant [15].

In South Africa, the B.1.351 variant emerged independently of B.1.1.7. Originally detected in early October 2020, B.1.351 shares some mutations with B.1.1.7. Cases caused by this variant were reported in the US at the end of January 2021 [16]. As of March 2021, the B.1.1.7 variant was the most abundant virus

found in clinical samples of COVID-19 patients around the world, and the B.1.351 variant was the second most abundant [17].

The Delta B.1.617.2 variant, dominant in the powerful 2021 surge in COVID-19 cases in India, is currently rapidly spreading across the globe, threatening to overwhelm healthcare systems and negatively impact already challenged economies. While the data are still scarce, preliminary evidence questions the effectiveness of commercial vaccines in protecting against Delta B.1.617.2 transmission and associated morbidity.

Taffix® is used worldwide as an additional layer of protection to help reduce the risk of contracting viral diseases caused by viruses whose first portal of entry to the body passes through the nasal cavity, such as influenza viruses, rhinoviruses and coronaviruses. Its efficacy against the newly emerging SARS-CoV-2 variants is therefore clinically relevant. This study showed that *Taffix®* effectively blocks passage of the new variants under *in vitro* conditions [18, 19].

The concept of nonspecific protection against upper respiratory infectious viruses was first described by Hull [20] *et al*., who emphasized the potential of creating a hostile microenvironment in the nasal cavity to inhibit upper respiratory infectious pathogens. Indeed, many upper respiratory viruses are sensitive to low pH, including rhinoviruses [21, 22], influenza viruses [23], respiratory syncytial virus (RSV) [24], and coronavirus [25]. Hull *et al.* demonstrated in a randomized, double blind, placebo-controlled clinical study that irrigation of the nasal mucosa with an acidic hydrogel spray reduced the severity and duration of common cold symptoms [21]. Sungnak et al. [5] found that SARS-CoV-2 entry factors are highly expressed in goblet and ciliated nasal epithelial cells, together with innate immune genes. These factors render the nose a likely coronavirus entry point.

Taffix® low-pH (pH 3.5) gel forms a protective layer within 1 minute from spray and the acidic film is maintained on the nasal tissue for about 5 hours [8]. The protective layer mechanically prevents virus particles from reaching the nasal mucosa and creates hostile environment to pathogens due to the acidic pH. The natural pH of the human nasal mucosa is approximately 5.5-6.5 [24], which is a favorable environment for most viral pathogens including the SARS-COV-2 virus [24]. Thus, a protective device acting within a lower pH range is clinically advantageous. *Taffix®* is non-toxic, biocompatible, and can be easily used in addition to other protective measures, such as a face mask. It may also provide protection which is easy and comfortable to apply in situations where use of a face mask is not practical, for example during dining or sports activities.

Materials And Methods

2.1. Materials

Alpha virus stock was isolated from patients in Israel on 20 December 2020. Beta virus stock was isolated from patients in Israel on 15 January 2021. Delta virus stock was isolated from patients in Israel on 20 April 2021. Cell strainers (40 µm Cat# SO SCS402) were obtained from AlexRed, cell culture

medium (MEM-EAGLE) was obtained from BI Beit Haemek, Vero-E6 cells were obtained from HTCC and *TaffiX®* was supplied by Nasus Pharma Ltd.

2.2. Virus propagation

Vero-E6 cells were grown at 37 °C, 5% CO_2 in MEM-Eagle Earle's salts Base (Biological Industries, Israel) containing 50 IU/ml penicillin and 50 µg/ ml streptomycin (Biological industries, Israel) and 10% heat-inactivated fetal calf serum (FCS) (Biological Industries, Israel). Confluent Vero-E6 cells were incubated for 1 h at 33□C with 300 µl of the nasopharyngeal samples stored in Copan Universal Transport Medium (UTM-RT, Italy), after which, 5 ml MEM-EAGLE medium supplemented with 2% fetal calf serum (FCS) were added. The infected cells were cultured for 3-10 days. When a cytopathic effect (structural changes in host cells) was observed, 300 µl of the supernatant was added to T-75 flasks seeded with $10x10^6$ Vero-E6 cells, to which 20 ml 2% FCS MEM-EAGLE were immediately added. Samples were incubated at 33□C for 3-5 days, in order to reach higher viral loads. When a cytopathic effect was microscopically observed, supernatants were aliquoted and stored at -80□C.

2.3. Taffix® assay

Sterile water (150 ml) was added to sterile 40 mm cell strainers placed on a 50 ml tube. *Taffix®* (20 mg) was then added and mixed with the water with a pipette tip until a homogeneous layer formed across the cell strainer surface. Filters were then incubated at room temperature for 10 min. Control filters were incubated with 150 ml sterile water only. Thereafter, 10 µl of SARS-CoV-2 Alpha, Beta or Delta variants were seeded on the filters at a concentration CT 20 (approximately ~600,000 copies/ml) for Alpha and Beta, and CT 21 (approximately ~300,000 copies/ml) for Delta, which is parallel to the clinical concentrations found in nasal swabs of patients diagnosed positive for SARS-CoV-2 (COVID-19).

After a 10-min incubation at room temperature, the downstream side of each filter was washed with 500 µl cell culture medium, which was subsequently collected and diluted 1:10 with culture medium. A sample was taken from the rinse solution of the Delta variant for determination of viral count using qRT-PCR. Then, 50 µl of the diluted medium was placed into 24-well plates containing Vero-E6 cells ($2x10^5$ cells/well, in 2% fetal calf serum). After 5 days of incubation at 33°C (5% CO_2), a 200 µl sample from each well was taken for viral RNA extraction followed by qRT-PCR analysis. Each treatment was repeated in duplicates for Alpha and Beta variants and in triplicates for the Delta variant.

2.4. Quantitative reverse transcriptase PCR

Nucleic acids were extracted using the MagLead extractor (PSS, Japan), according to the manufacturer's instructions. Reverse transcription polymerase chain reactions (RT-PCRs) using primers corresponding to the SARS-CoV-2 envelope (E) gene, were performed as previously described [27]. Quantitative RT-PCR (qRT-PCR) was performed in 25 µl SensiFast reaction mix (Bioline, USA) using TaqMan Chemistry on a CFX-96 instrument.

Conclusions

In conclusion, under in vitro conditions, *Taffix®* formed a highly effective protective barrier against SARS-COV-2 variants. These results are consistent with prior findings demonstrating the high *in vitro* efficacy of *Taffix®* gel in preventing viruses from reaching and infecting cells, and support the use of nasal spray as an effective barrier against new variants of SARS-CoV-2. Since the development and distribution of new vaccines against novel variants is a long and complex process, additional protective measures are required. *Taffix®* may provide a simple and safe means of reducing infections and spread of COVID-19.

Declarations

Author Contributions: Conceptualization, Tair Lapidot and Dalia Megiddo; methodology, Micha Gladnikoff, Michal Mandelboim, and Yaron Drori; validation and experimentation, Nofar Atari; writing—original draft preparation Tair Lapidot and Dalia Megiddo; writing—review and editing, Michal Mandelboim, Ella Mendelson; supervision, Ella Mendelson and Tair Lapidot. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this manuscript are available on request from the corresponding author.

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Figures

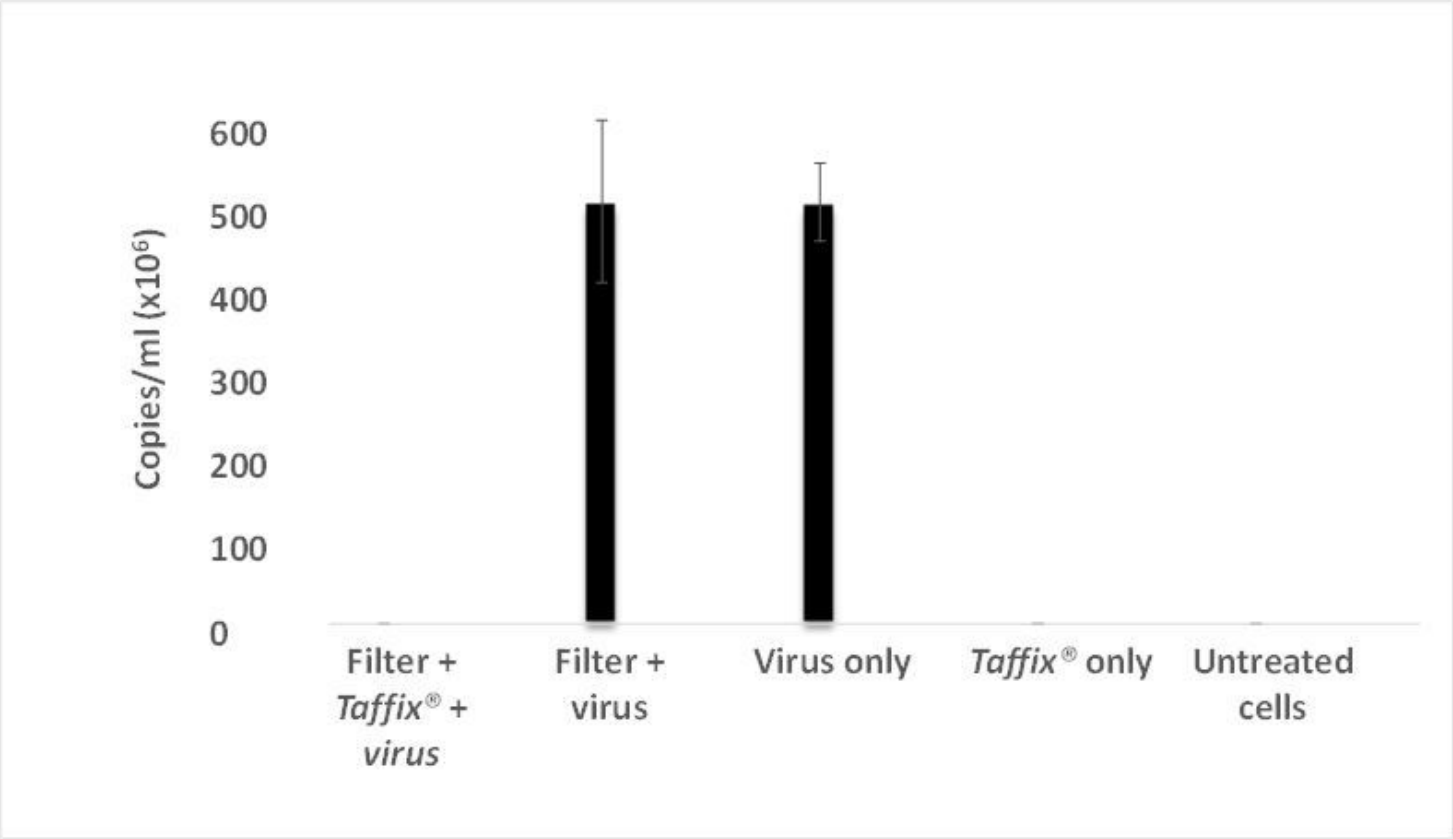

Figure 1

Taffix® prevented infection of Vero-E6 cells by the Alpha B.1.1.7 SARS-CoV-2 variant. Vero-E6 cells were incubated with inoculum of SARS-CoV-2 variant Alpha B.1.1.7 at a concentration of ~600,000 copies/ml (which is parallel to a clinical PCR results of CT20), which had been passed through an untreated cell strainer filter or through one pretreated with Taffix®. Total RNA was extracted from the growth medium 5 days later for qPCR analysis. Viral copies were calculated from qPCR CT values. For each test, a positive sRNA control and negative control were used. The results are average of duplicates.

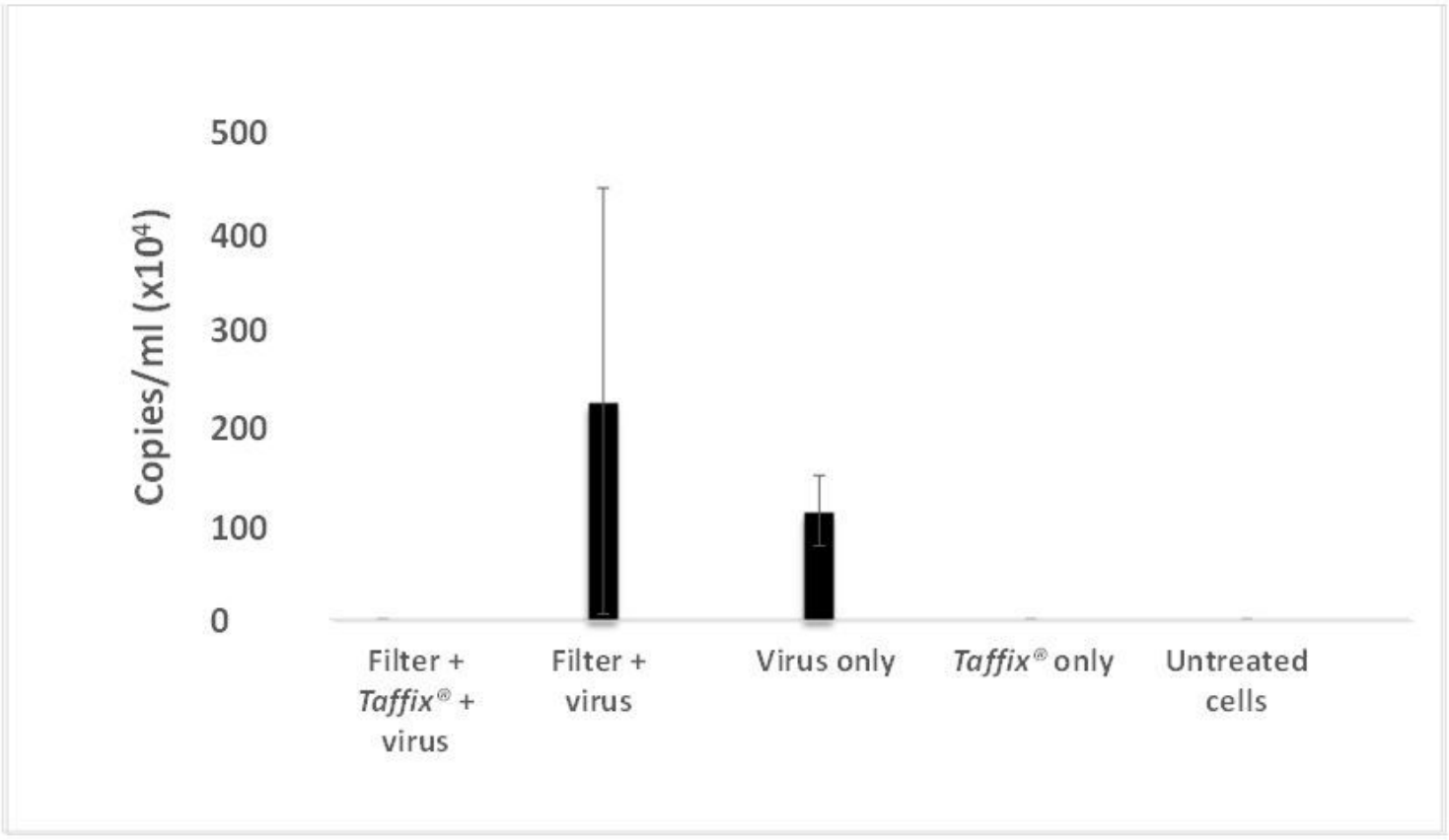

Figure 2

Taffix prevented infection of Vero-E6 cells by the Beta B.1.351 SARS-CoV-2 variant. Vero-E6 cells were grown and incubated with inoculum of SARS-CoV-2 variant Beta B.1.351 at a concentration of ~600,000 copies/ml (which is parallel to a clinical PCR result of CT20), which had been passed through an untreated cell strainer filter or through one pretreated with Taffix®. Total RNA was extracted from the growth medium 5 days later for qPCR analysis. Viral copies were calculated from qPCR CT values. For each test, a positive sRNA control and negative control were used. The results are average of duplicates.

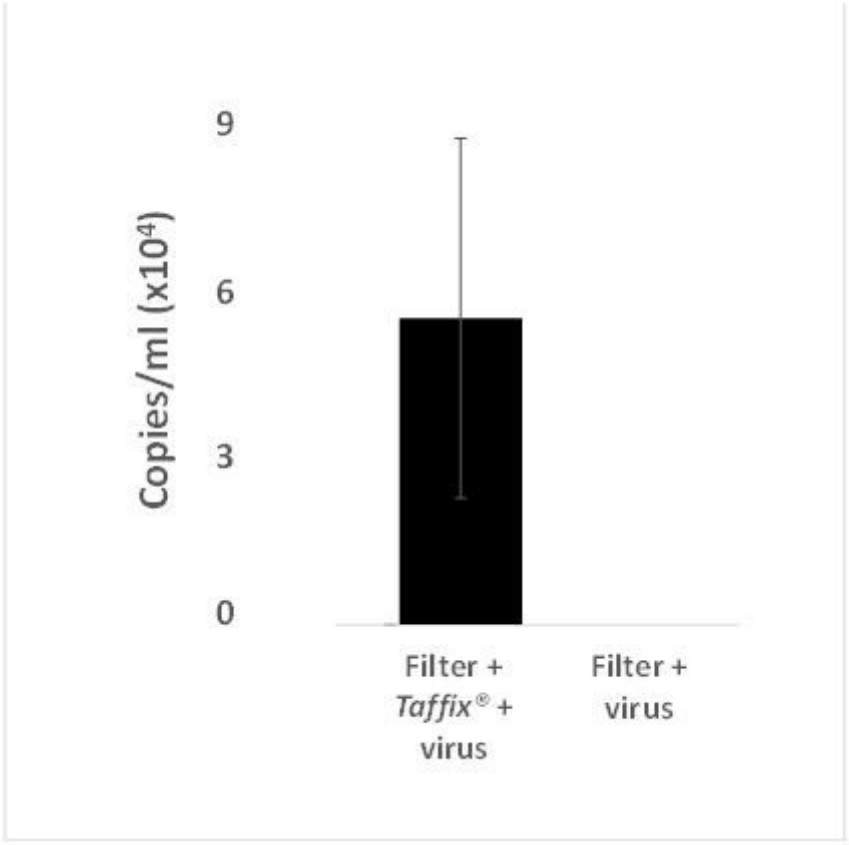

Figure 3

Taffix prevented infection of Vero-E6 cells by the Delta B.1.617.2 SARS-CoV-2 variant. Vero-E6 cells were grown and incubated with inoculum of SARS-CoV-2 variant Delta B.1.617.2 at a concentration of ~300,000 copies/ml (which is parallel to a clinical PCR result of CT21), which had been passed through an untreated cell strainer filter or through one pretreated with Taffix®. Total RNA was extracted from the growth medium 5 days later for qPCR analysis. Viral copies were calculated from qPCR CT values. For each test, a positive sRNA control and negative control were used. The results are average of triplicates.